Saliva-Extracted DNA is a Noninvasive Sampling Technique for Examining OPRM1 Gene Methylation in Preterm Infants

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Background: All chronic pain was once undermanaged acute pain.¹ Chronic pain is a significant problem affecting 20% to 35% of children around the world.² Undermanaged acute pain early in life is the greatest risk factor for the development of chronic pain persisting into adulthood.³⁻⁵ The traditional methods for studying pediatric chronic pain does not predict who will develop chronic pain and who will recover uneventfully. Genetic-epigenetic interactions reducing OPRM1 µ opioid receptor expression may partially explain the transition from acute to chronic pain.⁶ During normal development, infant pain transmission and modulation undergo rapid growth beginning at 22 weeks gestation achieving mature functioning at 2 months of age.⁷ Data demonstrate acute injury from noxious stimuli during this critical vulnerable period of neuronal plasticity may trigger unpredicted long-term epigenetic changes, such a gene methylation, which affects brain, neurodevelopment, pain modulation and pain reactivity that persists into adulthood.⁸ Gene methylation in the µ opioid receptor OPRM1 may be partially responsible for the transition from acute to chronic pain in children.

The clinical standard for genetic testing is invasive phlebotomy. However, repeated blood draws can lead to anemia, blood transfusions, cardiorespiratory instability and exposure to noxious stimuli in this vulnerable population.⁹ Saliva-extracted collection procedures would vastly improve the quality of care for these infants and positively affect their long-term clinical outcomes; but concerns over immature saliva development, minute saliva volumes, lower mean DNA yield, mode of birth, initiation of feeds, type of nutrition and greater contamination with bacterial DNA challenge the suitability of saliva extracted DNA in premature infants.⁹,¹⁰

Purpose: To determine the effectiveness of a noninvasive DNA sampling technique for examining epigenetic modifications in preterm infants.

Methods:  
Design: Within subject change over time candidate gene DNA methylation association study.  
Setting/Sample: Urban teaching hospital’s neonatal intensive care unit and newborn nursery. Convenience sample of healthy full-term (>37 weeks, n=6) and preterm (<37 weeks, n=6) infants.  
Procedure: Infant buccal saliva was collected after the infant’s admission to the newborn nursery or neonatal intensive care unit and prior to the infant’s discharge.  
Analysis: The methylation pattern at the 5’ end of OPRM1 was examined. DNA was treated with bisulphite to convert all cytosines to uracil residues, leaving methylated cytosines unchanged. Sequencing of bisulfite-converted DNA was carried out. The
sequence of bisulfite converted DNA clones were aligned with ClustalW, fidelity of the polymerase chain reaction and the sequencing reaction evaluated, and the methylation pattern identified.

**Results:** Sequence analysis of four overlapping clones within exon 1 of **OPRM1** revealed thirteen CpG sites methylated in more than 50% of the clones sequenced. Preterm infant and control DNA displayed similar methylation patterns at these sites. Two CpG sites showed a higher percentage of methylation in preterm infant DNA samples compared to controls, but in less than 50% of the clones sequenced.

**Conclusion:** Saliva-extracted DNA is a feasible, noninvasive DNA sampling technique for examining OPRM1 gene methylation in preterm infants. Interventions to mitigate pain in early life may prevent epigenomic changes that increase an individual's susceptibility for developing chronic pain.

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**Keywords:**
Saliva-extracted DNA, gene methylation and preterm infants

**Abstract Summary:**
Mitigating pain in early life may prevent epigenetic changes that increase an individual's susceptibility for developing chronic pain. Adverse effects and the quality of the DNA collected challenge the suitability of saliva-extracted DNA. Saliva-extracted DNA is a feasible, noninvasive DNA sampling technique for examining OPRM1 gene methylation in preterm infants.

**References:**

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Author Summary: Dr. Hatfield is a respected researcher, professor, and mentor to students, clinical nurses and other professionals. Her program of research investigating the analgesic properties of behavioral and environmental interventions, epigenetic modifications following noxious stimuli, the interactions between undermanaged pain and infection, the long-term effects of undermanaged pain in infants and the transition of acute to chronic pain in early life has received national and international recognition.

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